

=> d his

(FILE 'HOME' ENTERED AT 13:27:04 ON 15 APR 2004)

FILE 'CAPLUS' ENTERED AT 13:27:12 ON 15 APR 2004

```
L1      1 S ASTAXANTHIN SYNTHASE
        SET SMA OFF
        SEL RAN.CAPLUS(1) L1 1
        SET SMA LOGIN
L2      1 S E1
        SET SMA OFF
        SEL RAN.CAPLUS(2) L1 1
        SET SMA LOGIN
L3      1 S E2
        SET SMA OFF
        SEL RAN.CAPLUS(3) L1 1
        SET SMA LOGIN
L4      1 S E3
L5      78943 S SYNTHASE
L6      63109 S ACTIVE SITE
L7      1859 S L5 AND L6
L8      17192 S BETA CAROTENE
L9      0 S L7 AND L8
L10     2097 S ASTAXANTHIN
L11     854 S L8 AND L10
L12     1 S L1 AND L11
L13     10 S AST GENE
L14     0 S L5 AND L13
L15     0 S L8 AND L14
L16     337 S RHODOZYMA
L17     78949 S SYNTHASE?
L18     9 S L16 AND L17
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changes
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NEWS 7 MAR 03 MEDLINE file segment of TOXCENTER reloaded
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=> file caplus

COST IN U.S. DOLLARS

SINCE FILE

TOTAL

ENTRY

SESSION

FULL ESTIMATED COST

0.21

0.21

FILE 'CAPLUS' ENTERED AT 13:27:12 ON 15 APR 2004

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FILE COVERS 1907 - 15 Apr 2004 VOL 140 ISS 16
FILE LAST UPDATED: 14 Apr 2004 (20040414/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

=> s astaxanthin synthase
2097 ASTAXANTHIN
(ASTAXANTHIN OR ASTAXANTHINS)
78943 SYNTHASE
(SYNTHASE OR SYNTHASES)
L1 1 ASTAXANTHIN SYNTHASE
(ASTAXANTHIN(W)SYNTHASE)

=> d all

L1 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:645717 CAPLUS
DN 133:234467
ED Entered STN: 15 Sep 2000
TI Cloning and sequence of **astaxanthin synthase** from
Phaffia rhodozyma and use of the enzyme for production of astaxanthin
IN Hoshino, Tatsuo; Ojima, Kazuyuki; Setoguchi, Yutaka
PA F. Hoffmann-La Roche A.-G., Switz.
SO Eur. Pat. Appl., 46 pp.
CODEN: EPXXDW
DT Patent
LA English
IC ICM C12N015-52
ICS C12N009-00; C12P023-00
CC 7-5 (Enzymes)

Section cross-reference(s): 3, 10, 16

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 1035206	A1	20000913	EP 2000-104430	20000303
	EP 1035206	B1	20031015		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	US 6365386	B1	20020402	US 2000-518386	20000303
	AT 252155	E	20031115	AT 2000-104430	20000303
	CN 1266101	A	20000913	CN 2000-103755	20000308
	BR 2000001369	A	20010814	BR 2000-1369	20000308
	JP 2000262294	A2	20000926	JP 2000-65041	20000309
	US 2003077691	A1	20030424	US 2002-66007	20020201
PRAI	EP 1999-104668	A	19990309		
	EP 2000-101666	A	20000201		
	US 2000-518386	A3	20000303		
AB	The present invention is directed to genetic materials useful for the				

preparation of astaxanthin from β -carotene, such as polypeptides having **astaxanthin synthase** activity, DNA fragments coding for **astaxanthin synthase**, recombinant organisms and the like. Those novel genetic materials may be originated from *Phaffia rhodozyma*. Cloning, genomic and cDNA sequences of **astaxanthin synthase** of *P. rhodozyma* and amino acid sequence of the encoded enzyme are disclosed. The present invention also provides a process for the production of astaxanthin.

- ST *Phaffia* **astaxanthin synthase** gene cDNA sequence;
astaxanthin prodn synthase *Phaffia*
- IT Culture media
DNA sequences
Electron donors
Fermentation
Molecular cloning
Phaffia rhodozyma
Protein sequences
cDNA sequences
(cloning and sequence of **astaxanthin synthase** from
Phaffia rhodozyma and its use for production of astaxanthin)
- IT Genetic element
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(exon; cloning and sequence of **astaxanthin synthase**
from *Phaffia rhodozyma* and its use for production of astaxanthin)
- IT Gene, microbial
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(for **astaxanthin synthase**; cloning and sequence of
astaxanthin synthase from *Phaffia rhodozyma* and its
use for production of astaxanthin)
- IT Genetic element
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(intron; cloning and sequence of **astaxanthin synthase**
from *Phaffia rhodozyma* and its use for production of astaxanthin)
- IT Mitochondria
Mitochondria
(membrane, reconstituted **astaxanthin synthase** in
membrane like; cloning and sequence of **astaxanthin synthase** from *Phaffia rhodozyma* and its use for production of
astaxanthin)
- IT Membrane, biological
Membrane, biological
(mitochondrial, reconstituted **astaxanthin synthase**
in membrane like; cloning and sequence of **astaxanthin synthase** from *Phaffia rhodozyma* and its use for production of
astaxanthin)
- IT Microsome
(reconstituted **astaxanthin synthase** in membrane
like; cloning and sequence of **astaxanthin synthase**
from *Phaffia rhodozyma* and its use for production of astaxanthin)
- IT Liposomes
Membrane, biological
(reconstituted **astaxanthin synthase** in; cloning and
sequence of **astaxanthin synthase** from *Phaffia rhodozyma* and its use for production of astaxanthin)
- IT 292666-76-3P
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); CAT (Catalyst use); PRP (Properties); BIOL (Biological study); PREP (Preparation); USES (Uses)
(amino acid sequence; cloning and sequence of **astaxanthin synthase** from *Phaffia rhodozyma* and its use for production of

astaxanthin)

IT 293749-46-9P, **Astaxanthin synthase**
 RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); CAT (Catalyst use); PRP (Properties); BIOL (Biological study); PREP (Preparation); USES (Uses)
 (cloning and sequence of **astaxanthin synthase** from *Phaffia rhodozyma* and its use for production of astaxanthin)

IT 9039-06-9, Cytochrome P 450 reductase
 RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); CAT (Catalyst use); BIOL (Biological study); USES (Uses)
 (cloning and sequence of **astaxanthin synthase** from *Phaffia rhodozyma* and its use for production of astaxanthin)

IT 472-61-7P, Astaxanthin
 RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PREP (Preparation)
 (cloning and sequence of **astaxanthin synthase** from *Phaffia rhodozyma* and its use for production of astaxanthin)

IT 7235-40-7, β -Carotene
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
 (cloning and sequence of **astaxanthin synthase** from *Phaffia rhodozyma* and its use for production of astaxanthin)

IT 292666-77-4 292885-96-2, 2: PN: EP1035206 SEQID: 3 claimed DNA
 RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
 (nucleotide sequence; cloning and sequence of **astaxanthin synthase** from *Phaffia rhodozyma* and its use for production of astaxanthin)

IT 292666-90-1, 4: PN: EP1035206 SEQID: 8 unclaimed DNA 292666-91-2, 5: PN: EP1035206 SEQID: 9 unclaimed DNA 292666-92-3, 6: PN: EP1035206 SEQID: 10 unclaimed DNA 292666-93-4, 7: PN: EP1035206 SEQID: 11 unclaimed DNA 292666-94-5, 8: PN: EP1035206 SEQID: 12 unclaimed DNA 292666-95-6, 9: PN: EP1035206 SEQID: 13 unclaimed DNA 292666-96-7 292666-97-8 292666-98-9 292666-99-0 292667-00-6 292667-01-7 292667-02-8 292667-03-9 292667-04-0 292667-05-1 292667-06-2 292667-07-3 292667-08-4 292667-09-5 292667-10-8 292667-11-9 292667-12-0 292667-13-1 292667-14-2, 29: PN: EP1035206 SEQID: 4 unclaimed DNA 292667-15-3, 1: PN: EP1035206 SEQID: 5 unclaimed DNA 292667-16-4, 2: PN: EP1035206 SEQID: 6 unclaimed DNA 292667-17-5, 3: PN: EP1035206 SEQID: 7 unclaimed DNA
 RL: PRP (Properties)
 (unclaimed nucleotide sequence; cloning and sequence of **astaxanthin synthase** from *Phaffia rhodozyma* and use of the enzyme for production of astaxanthin)

RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
 RE
 (1) Gist Brocades Bv; WO 9723633 A 1997 CAPLUS
 (2) Kirin, B; EP 0769551 A 1997 CAPLUS
 (3) Wery, J; GENE 1997, V184(1), P89 CAPLUS

=> SET SMA OFF

SET COMMAND COMPLETED

=> SEL RAN.CAPLUS(1) L1 1

E1 THROUGH E1 ASSIGNED

=> SET SMA LOGIN

SET COMMAND COMPLETED

=> S E1

L2 1 "1997:506702"/AN

=> D L2 BIB,ABS

L2 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN

AN 1997:506702 CAPLUS

DN 127:145923

TI Improved transformation of and expression in Phaffia by using the promoter of glycolytic pathway gene or ribosomal protein gene

IN Verdoes, Jan Cornelis; Wery, Jan

PA Gist-Brocades B.V., Neth.; Ooijen, Albert Johannes Joseph; Verdoes, Jan Cornelis; Wery, Jan

SO PCT Int. Appl., 117 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9723633	A1	19970703	WO 1996-EP5887	19961223
	W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
	RW:	KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
	EP 780474	A1	19970625	EP 1995-203620	19951222
	R: NL				
	CA 2241267	AA	19970703	CA 1996-2241267	19961223
	AU 9713087	A1	19970717	AU 1997-13087	19961223
	AU 725340	B2	20001012		
	EP 870042	A1	19981014	EP 1996-944694	19961223
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2000507087	T2	20000613	JP 1997-523340	19961223
	US 6329141	B1	20011211	US 1998-91725	19981119
PRAI	EP 1995-203620	A	19951222		
	EP 1996-200943	A	19960411		
	WO 1996-EP5887	W	19961223		

AB The transformation efficiency of and expression level in Phaffia can be improved by using the high-level promoter of a glycolytic pathway gene (e.g. glyceraldehyde-3-phosphate dehydrogenase (gpd)) or a ribosomal protein gene (e.g. 40S ribosomal protein S27). High level expression of a carotenoid biosynthetic pathway gene in Phaffia rhodozyma may be obtained by using an expression vector containing one of the above promoters and the gene gpd terminator/polyadenylation site. Also disclosed are the cDNA encoding the enzymes involved in the carotenoid biosynthetic pathway of Phaffia rhodozyma: isopentenyl diphosphate isomerase (idi), geranylgeranyl pyrophosphate synthase (crtE), phytoene synthase (crtB), phytoene desaturase (crtI), and lycopene cyclase (crtY). Heterologous expression of carotenogenic genes from Erwinia uredovora in P. rhodozyma by using an expression vector containing the gene gpd promoter was demonstrated. Isolation of carotenogenic genes by heterologous hybridization from carotenogenic fungi was also shown. Use of the vectors, transformed P. rhodozyma for making proteins and/or carotenoids (e.g. astaxanthin), and methods for isolating highly expressed promoters from Phaffia are also claimed.

=> d kwic

L2 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1997:506702 CAPLUS
DN 127:145923

=> SET SMA OFF

SET COMMAND COMPLETED

=> SEL RAN.CAPLUS(2) L1 1

E2 THROUGH E2 ASSIGNED

=> SET SMA LOGIN

SET COMMAND COMPLETED

=> S E2

L3 1 "1996:659426"/AN

=> D L3 BIB,ABS

L3 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1996:659426 CAPLUS
DN 125:294752
TI Recombinant preparation of carotenoid in transgenic microorganisms with improved yield
IN Kajiwara, Susumu; Misawa, Norihiko; Kondo, Keiji
PA Kirin Beer Kabushiki Kaisha, Japan
SO PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DT Patent
LA Japanese
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	-----		-----	-----	-----
PI	WO 9628545	A1	19960919	WO 1996-JP574	19960308
	W: AU, NO, US				
	RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	JP 08242861	A2	19960924	JP 1995-51234	19950310
	JP 3151371	B2	20010403		
	JP 2001136992	A2	20010522	JP 2000-320990	19950310
	JP 3403381	B2	20030506		
	AU 9648899	A1	19961002	AU 1996-48899	19960308
	AU 685354	B2	19980115		
	EP 769551	A1	19970423	EP 1996-905037	19960308
	R: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
	NO 9604754	A	19970108	NO 1996-4754	19961108
PRAI	JP 1995-51234	A	19950310		
	WO 1996-JP574	W	19960308		

AB The cDNA encoding isopentenylpyrophosphate (IPP) isomerase were isolated from astaxanthin-producing *Phaffia rhodozyma* and *Haematococcus pluvialis* and their amino acid sequences deduced. The sequences were highly similar to that of *Saccharomyces cerevisiae*. Transformation of the IPP isomerase-encoding cDNA into *Escherichia coli* strain JM101 that was already a lycopene producer further increased its yield (3.6-4.5 folds). This process makes it possible to significantly increase of the yield of

carotenoids, useful food/feed coloring agents and therapeutics, in transgenic microorganisms.

=> SET SMA OFF

SET COMMAND COMPLETED

=> SEL RAN.CAPLUS(3) L1 1

E3 THROUGH E3 ASSIGNED

=> SET SMA LOGIN

SET COMMAND COMPLETED

=> S E3

L4 1 "1996:737301"/AN

=> D L4 BIB,ABS

L4 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN

AN 1996:737301 CAPLUS

DN 126:99964

TI High copy number integration into the ribosomal DNA of the yeast *Phaffia rhodozyma*

AU Wery, Jan; Gutker, Diana; Renniers, Anton C. H. M.; Verdoes, Jan C.; van Ooyen, Albert J. J.

CS Division of Industrial Microbiology, Department of Food Science, Wageningen Agricultural University, P.O. Box 8129, 6700 EV, Wageningen, Neth.

SO Gene (1997), 184(1), 89-97

CODEN: GENED6; ISSN: 0378-1119

PB Elsevier

DT Journal

LA English

AB This report describes a transformation system leading to stable high copy number integration into the ribosomal DNA (rDNA) of the astaxanthin-producing yeast *Phaffia rhodozyma*. A plasmid was constructed that contains the transposon Tn5 encoded kanamycin resistance gene (KmR) fused in frame to the 5'-terminal portion of the *Phaffia* actin gene. This marker, driven by the *Phaffia* actin promoter, confers resistance to G418 (Geneticin). The plasmid also contains a rDNA portion that comprises the 18S rDNA and promotes high copy integration leading to stable *Phaffia* transformants that maintained the plasmid at high copy number after 15 generations of non-selective growth. *Phaffia*, strain CBS 6938, was found to contain the rDNA units in clusters distributed over three chromosomes with a total copy number of 61. *Phaffia* transformants were shown to have over 50 copies of pGB-Ph9 integrated in tandem in chromosomes that contain rDNA loci. The chromosomal shifts that occur as a result of these integrations as shown by pulsed field electrophoresis strongly suggest that *Phaffia* is haploid.

RE.CNT 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d his

(FILE 'HOME' ENTERED AT 13:27:04 ON 15 APR 2004)

FILE 'CAPLUS' ENTERED AT 13:27:12 ON 15 APR 2004

L1 1 S ASTAXANTHIN SYNTHASE


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                SET SMA OFF
                SEL RAN.CAPLUS(1) L1 1
                SET SMA LOGIN
L2              1 S E1
                SET SMA OFF
                SEL RAN.CAPLUS(2) L1 1
                SET SMA LOGIN
L3              1 S E2
                SET SMA OFF
                SEL RAN.CAPLUS(3) L1 1
                SET SMA LOGIN
L4              1 S E3

```

```

=> s synthase
L5              78943 SYNTHASE
                (SYNTHASE OR SYNTHASES)

```

```

=> s active site
                820432 ACTIVE
                (ACTIVE OR ACTIVES)
                833387 SITE
                (SITE OR SITES)
L6              63109 ACTIVE SITE
                (ACTIVE(W)SITE)

```

```

=> s l5 and l6
L7              1859 L5 AND L6

```

```

=> s beta carotene
                1247560 BETA
                (BETA OR BETAS)
                37302 CAROTENE
                (CAROTENE OR CAROTENES)
L8              17192 BETA CAROTENE
                (BETA(W)CAROTENE)

```

```

=> s l7 and l8
L9              0 L7 AND L8

```

```

=> s astaxanthin
L10             2097 ASTAXANTHIN
                (ASTAXANTHIN OR ASTAXANTHINS)

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=> s l8 and l10
L11             854 L8 AND L10

```

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=> d 850-854 ibib ab

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L11 ANSWER 850 OF 854 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1948:39426 CAPLUS
DOCUMENT NUMBER: 42:39426
ORIGINAL REFERENCE NO.: 42:8352g-i,8353a
TITLE: Astaxanthin in insects and other terrestrial
arthropods
AUTHOR(S): Manunta, C.
CORPORATE SOURCE: Univ. Sassari, Italy
SOURCE: Nature (London, United Kingdom) (1948), 162, 298
CODEN: NATUAS; ISSN: 0028-0836
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB The statement of Goodwin and Srisukh (C.A. 42, 3864i) that
astaxanthin was found by them for the 1st time in land animals is
erroneous. M. found it in a mite (Trombidium spp.) (C.A. 34, 175.1). It
was completely extracted by acetone from the mite body, transferred to

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petr.-ether, which on evaporation of solvent yielded an oily residue (3 g. mites gave 2 g. this oil). After saponification, the pigment was dissolved in benzene, and purified by chromatography, yielding red-violet crystals. These crystals gave all the reactions of carotenoids and a peculiar adsorption spectrum (single band at 515 m μ in CS₂ and at 500 m μ in pyridine). The tests show undoubted **astaxanthin**. A similar pigment was found recently in the Colorado potato beetle (*Leptinotarsa decemlineata*). The benzene fraction of this pigment gives with benzene at 70-80° an orange-yellow zone that splits to 2 zones; an epiphasic zone, rose-yellow consisting of **.beta.-carotene**; an epiphasic zone that progresses slowly on the column, eluted with benzene at 70-80° which shows rose-yellow, rose in CS₂, with a spectrum revealing one large band with maximum near 497 m μ . This pigment looks like one extracted and crystallized from the fat of *Phoenicopterus roseus* and named **phaenicoxanthin** (C.A. 34, 175.1). It shows one adsorption band near 490 m μ in pyridine and differs from astacene in position of adsorption band, in solution, and in color in various solvents. The metabolism of carotenoid pigments in *L. decemlineata* fed on various solanaceous plants is being studied.

L11 ANSWER 851 OF 854 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1948:23744 CAPLUS
 DOCUMENT NUMBER: 42:23744
 ORIGINAL REFERENCE NO.: 42:5126g-i
 TITLE: Investigations of gamones of the rainbow trout
 AUTHOR(S): Hartmann, Max; Medem, Fred Graf; Kuhn, Richard; Bielig, Hans-Joachim
 CORPORATE SOURCE: Kaiser-Wilhelm-Inst., Germany
 SOURCE: Zeitschrift fuer Naturforschung (1947), 2b, 330-49
 CODEN: ZNTFA2; ISSN: 0372-9516
 DOCUMENT TYPE: Journal
 LANGUAGE: Unavailable

AB Biol. methods for the detection of androgamone AI and AII and gynogamone GI and GII are described. Eggs of *Salmo irideus* contain **astaxanthin**, **.beta.-carotene**, lutein, and lactoflavin as pigments. **Astaxanthin**, C₄₀H₅₂O₄, m. 216°, has GI activity. It activates and acts chemotactically on rainbow trout sperm and is antagonistic to AI contained in the sperm. From the eggs was isolated a thermolabile protein-phosphatide GII which agglutinates spermatozoa in a dilution as high as 1:5,000,000,000. All of the sperm is thermolabile and antagonizes the action of the agglutinin.

L11 ANSWER 852 OF 854 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1948:17970 CAPLUS
 DOCUMENT NUMBER: 42:17970
 ORIGINAL REFERENCE NO.: 42:3864h-i,3865a-c
 TITLE: Carotenoids of the locust integument
 AUTHOR(S): Goodwin, T. W.; Srisukh, S.
 CORPORATE SOURCE: Univ., Liverpool, UK
 SOURCE: Nature (London, United Kingdom) (1948), 161, 525-6
 CODEN: NATUAS; ISSN: 0028-0836
 DOCUMENT TYPE: Journal
 LANGUAGE: Unavailable

AB The integuments of the grasshoppers, *Locusta migratoria migratoroides* and *Schistocerca gregaria* bred in captivity on a grass diet, were examined for carotenoids. Methods are not given. **Astaxanthin** (C.A. 32, 7138.8, 9053.6) (I) was found in both species as shown by identical absorption spectra in petr. ether (40-60°), pyridine, and CS₂. A mixture of the pigments cannot be separated chromatographically. Locust I reacted with K-butoxide in vacuo to give the purplish blue enol salt and was converted to astacene by alkali in presence of O. All reactions showed that locust I was identical with I from lobster carapaces. I occurs in the free form and is not esterified. It is found in all

developmental stages of the grasshoppers up to sexual maturity and in the solitary and gregarious phases. It is the predominant carotenoid in the presexual stages. At sexual maturity, **.beta.-carotene**, which is always present in the fatty tissues, begins to accumulate in the hypodermis and I begins to disappear. No explanation is offered for this behavior but information is accumulating which suggests a relationship between carotenoid metabolism and sexual function. I is present in the eyes of both the mature and immature grasshoppers. Pigmentation of gonads and fatty tissues of these grasshoppers is due almost wholly to **.beta.-carotene**; a little α -carotene but no xanthophylls is present (cf. C.A. 37, 1516.8). Except for its detection in the retinas and eye coloring of birds (cf. Brockmann and Volker, Z. physiol. Chemical 235, 8(1935); C.A. 32, 1771.1), this is the 1st reported occurrence of I in land animals.

L11 ANSWER 853 OF 854 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1940:2879 CAPLUS

DOCUMENT NUMBER: 34:2879

ORIGINAL REFERENCE NO.: 34:457h-i,458a-e

TITLE: Distribution of **astaxanthin** in the animal and plant kingdom

AUTHOR(S): Kuhn, Richard; Stene, Jorgine; Sorensen, Nils Andreas

SOURCE: Ber. (1939), 72B, 1688-701

DOCUMENT TYPE: Journal

LANGUAGE: Unavailable

AB A table is given showing all sources, ranging from green algae to birds, from which crystalline astacin (I), m. 228° (cf. C. A. 32, 9053.6), has been isolated. Especially noteworthy is its occurrence in the green alga, *Haematococcus pluvialis* (II), for it has hitherto been generally regarded as an animal pigment. The euglena rhodone (III) isolated by Tischer (C. A. 33, 6361.9) from the spores of II and which he considered to be 4,6,4',6'-tetraketo-**.beta.-carotene**, isomeric with I, agrees in crystalline form and all other properties with I, but he states that it forms no phenazine with o-C₆H₄(NH₂)₂. Using the same material and procedure the authors obtained his ester A (IV), which with alcoholate in a high vacuum gave a blue-black solution immediately changing to red in air; the course of the changes, as regards time and color, was exactly like that of lobster **astaxanthin** (V), m. 216°, and its fat acid esters. As III itself, like I, gives no such color reaction but forms an orange-red K salt, it is evident the native IV cannot be an ester of a tetraketone. This made T.'s structure for III questionable, for no autoxidizable tetraenol corresponding to V can be derived from a 4,6,4',6'-tetraketone. The carotenoid obtained by alkaline saponification of

IV was

identical in all respects with the I from lobsters and gave with o-C₆H₄(NH₂)₂ the known phenazine, m. 222-3°. Debye-Scherrer pictures of the 2 pigments likewise showed no differences. T. regarded IV, which m. 100-1°, as a dipalmitate, but the synthetic dipalmitate of V m. 72°. As a matter of fact, IV is a mono ester; analysis, catalytic hydrogenation and colorimetric mol.-weight detns. indicate the acid residue contains 16 C atoms and 1 double bond. The synthetic monopalmitate of V m. 113.5-14.5° (corrected). Ester C was obtained in crystalline form, m. 34-6°, and proved to be a diester of V, probably with the same acid as in IV. Brockmann and Volker (C. A. 28, 4454.2) isolated I from the "roses" of pheasants. The authors, avoiding alkaline saponification, found that here, too, V and its esters are present exclusively. The food of the pheasant, both wild and in various zoos, is known quite exactly and contains no V, indicating that the pheasant is capable of synthesizing it from other carotenoids. Wald and Zussmann (C. A. 31, 7103.8) were able to obtain I, with other carotenoids, from the retina of chickens. The opinion already expressed that this was an artefact has been confirmed. The pigment isolated without the use of alkali gave with alcoholate the color reaction of V. Chromatographic analysis on sucrose showed the retina of the hen contains at least 2

different esters of V. V was obtained in 23.6-mg. yield from a liver (1080 g.) of *Regalecus glesne*. The synthetic dipalmitate of I m. 121°; the name astacein for the supposed dipalmitate in the lobster (Karrer, Loewe and Hubner, C. A. 29, 2544.1) is now superfluous.

L11 ANSWER 854 OF 854 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1938:64637 CAPLUS
DOCUMENT NUMBER: 32:64637
ORIGINAL REFERENCE NO.: 32:9053f-i,9054a-h
TITLE: **Astaxanthin** and ovoverdin
AUTHOR(S): Kuhn, Richard; Sorensen, Nils A.
SOURCE: Ber. (1938), 71B, 1879-88
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable

AB The green chromoprotein (I) in the eggs of *Astacus gammarus* is easily decomposed by alc., acetone, dilute acids or heat into a red pigment (II) which with alc. KOH gives astacin (III) (C. A. 27, 3530; 28, 217.1; Karrer and Hubner, C. A. 30, 6387.5). An interesting question was how combination of a red carotenoid with a colorless protein component can give a deep blue-green chromoprotein. II, originally designated ovo ester, is not an ester but a hydroxylated carotenoid C₄₀H₅₂O₄, i. e., a xanthophyll, and it is accordingly called **astaxanthin**. It differs from III in containing 4 more H atoms. In alkaline solution it uses up exactly 2 mols. O, smoothly giving III: II + 2O = III + 2H₂O₂. If O is strictly excluded, no trace of III is formed. The process hitherto thought to be a saponification is therefore really an autoxidation. On the basis

of the triketo-**beta**-carotene structure for III which the work of Karrer and his colleagues has made very probable, it may be concluded that II contains 2 secondary alc. groups in the place of 2 of the ketone groups in III. The HO groups can readily be detected by esterification. No tetraesters could be prepared; the keto groups in II do not enolize under the same conditions as those in III. With MeMgI II gives only 2 mols. CH₄ and its diacetate shows no active H at 20°. The absence of CH₂ groups next to the CO groups would explain why, unlike III, the distribution of II between benzene and aqueous MeOH is not influenced by dilute NaOH. It is very probable that the 2 CO groups are in conjugation with the polyene chain. II would then be a 5,5'-dihydroxy-4,4'-diketo-**beta**-carotene. Whereas III has only 1 homogeneous absorption band, II and its esters show 3 distinct maximum in the visible region. When O is strictly excluded, II gives deep blue alkali salts. If air is admitted the color immediately changes to red and III is formed. The phenomenon is similar to the formation of the orange K stilbenediolate (IV) from benzoin and K alcoholate. The blue salts are probably formed by double enolization and have the structure (R = polyene chain). They have not been isolated in analyzable form but on decomposition with dilute H₂SO₄ in

a

high vacuum they give II exclusively. Ovoverdin (I) is also assumed to be an analog of IV and is assigned a structure similar to that above, with basic groups of the protein component replacing the K atoms. This would explain its blue-green color. Unlike the blue salts, however, it is not autoxidizable; this is believed to be due to the fact that the protein is present not only in salt-like combination but that, as in the formation of flavoproteins and flavophosphoproteins, forces come into play which effect a sp., relatively firm "anchoring" of the pigment to the protein. From sedimentation studies of hardly purified solns. of I from the eggs. of *Homarus americanus*, Wyckoff (C. A. 31, 8568.6) obtained values corresponding to a mol. weight of about 300,000. The question was whether with increasing purification the ratio of II to protein in I would approach the value 1:500 corresponding to such a mol. weight. With fresh eggs. of North Sea lobsters as starting material, the content of II, after cleavage of the protein fraction with pyridine, was determined calorimetrically in a step photometer. The protein content was determined by precipitation

with tannin

(C. A. 32, 202.2) and Kjeldahl N detns. on the ppts. The I was purified by fractional adsorption on Al(OH)₃ and fractional elution with Na₂HPO₄ or 40%-saturated (NH₄)₂SO₄ under N in a refrigerator. There were thus obtained products with a constant ratio II: protein of 1:242. The absorption spectrum did not change during the course of the purification. The mol. weight determined in this way is therefore around 144,000. The epiphasic pigments

in the red epidermis of the lobster, hitherto considered to be esters of III, are really esters of II, for when the saponification is effected in the complete absence of air there are obtained the characteristic deeply colored salts of II which are instantly dehydrogenated to the tetraketone only when air is admitted. The chromoproteins also yield the double α -ketol with heat or dilute acids. The pigment of the boiled lobster is therefore II, not III. This is probably true of all Crustacea insofar as putrefaction or other factors have not set up an alkaline reaction which makes possible autoxidation to the tetraketone on boiling. II, m. 215-16° (decomposition), α 672.5 \pm 0.03° (7.22 mg. in 10 cc. pyridine, 1 2 dm.). Diacetate, deep blue-black, m. 203- 5° (evacuated tubes, Berl block), goes into the lower layer in distribution tests between 90% MeOH and benzene. Dicaprylate, dark red, m. 121-4° (in vacua, Berl block); only very little can be shaken out of benzene with 95% MeOH and less than half with 90% MeOH, but with 97% MeOH most of the pigment goes into the lower layer. Dipalmitate, violet-red, m. 71.5-2.5°.

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(FILE 'HOME' ENTERED AT 13:27:04 ON 15 APR 2004)

FILE 'CAPLUS' ENTERED AT 13:27:12 ON 15 APR 2004

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L1      1 S ASTAXANTHIN SYNTHASE
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L2      1 S E1
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        SEL RAN.CAPLUS(2) L1 1
        SET SMA LOGIN
L3      1 S E2
        SET SMA OFF
        SEL RAN.CAPLUS(3) L1 1
        SET SMA LOGIN
L4      1 S E3
L5      78943 S SYNTHASE
L6      63109 S ACTIVE SITE
L7      1859 S L5 AND L6
L8      17192 S BETA CAROTENE
L9      0 S L7 AND L8
L10     2097 S ASTAXANTHIN
L11     854 S L8 AND L10
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=> s l1 and l11

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L12     1 L1 AND L11
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L12 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:645717 CAPLUS

DOCUMENT NUMBER: 133:234467

TITLE: Cloning and sequence of **astaxanthin synthase** from *Phaffia rhodozyma* and use of the enzyme for production of **astaxanthin**

INVENTOR(S): Hoshino, Tatsuo; Ojima, Kazuyuki; Setoguchi, Yutaka

PATENT ASSIGNEE(S): F. Hoffmann-La Roche A.-G., Switz.
 SOURCE: Eur. Pat. Appl., 46 pp.
 CODEN: EPXXDW
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1035206	A1	20000913	EP 2000-104430	20000303
EP 1035206	B1	20031015		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
US 6365386	B1	20020402	US 2000-518386	20000303
AT 252155	E	20031115	AT 2000-104430	20000303
CN 1266101	A	20000913	CN 2000-103755	20000308
BR 2000001369	A	20010814	BR 2000-1369	20000308
JP 2000262294	A2	20000926	JP 2000-65041	20000309
US 2003077691	A1	20030424	US 2002-66007	20020201
PRIORITY APPLN. INFO.:			EP 1999-104668	A 19990309
			EP 2000-101666	A 20000201
			US 2000-518386	A3 20000303

AB The present invention is directed to genetic materials useful for the preparation of **astaxanthin** from **.beta.-carotene**, such as polypeptides having **astaxanthin synthase** activity, DNA fragments coding for **astaxanthin synthase**, recombinant organisms and the like. Those novel genetic materials may be originated from *Phaffia rhodozyma*. Cloning, genomic and cDNA sequences of **astaxanthin synthase** of *P. rhodozyma* and amino acid sequence of the encoded enzyme are disclosed. The present invention also provides a process for the production of **astaxanthin**.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s ast gene
 3441 AST
 (AST OR ASTS)
 899091 GENE
 (GENE OR GENES)
 L13 10 AST GENE
 (AST(W) GENE)

=> d his

(FILE 'HOME' ENTERED AT 13:27:04 ON 15 APR 2004)

FILE 'CAPLUS' ENTERED AT 13:27:12 ON 15 APR 2004

L1 1 S ASTAXANTHIN SYNTHASE
 SET SMA OFF
 SEL RAN.CAPLUS(1) L1 1
 SET SMA LOGIN
 L2 1 S E1
 SET SMA OFF
 SEL RAN.CAPLUS(2) L1 1
 SET SMA LOGIN
 L3 1 S E2
 SET SMA OFF
 SEL RAN.CAPLUS(3) L1 1
 SET SMA LOGIN
 L4 1 S E3
 L5 78943 S SYNTHASE
 L6 63109 S ACTIVE SITE

L7 1859 S L5 AND L6
 L8 17192 S BETA CAROTENE
 L9 0 S L7 AND L8
 L10 2097 S ASTAXANTHIN
 L11 854 S L8 AND L10
 L12 1 S L1 AND L11
 L13 10 S AST GENE

=> s l5 and l13

L14 0 L5 AND L13

=> s l8 and l14

L15 0 L8 AND L14

=> s rhodozyma

L16 337 RHODOZYMA
 (RHODOZYMA OR RHODOZYMAS)

=> s synthase?

L17 78949 SYNTHASE?

=> s l16 and l17

L18 9 L16 AND L17

=> d 1-9 ibib ab

L18 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2004:20837 CAPLUS

DOCUMENT NUMBER: 140:92684

TITLE: Genetically engineered Escherichia coli strains and
 fermentation process for production of glucosamine and
 N-acetylglucosamine

INVENTOR(S): Deng, Ming-de; Angerer, J. David; Cyron, Don; Grund,
 Alan D.; Jerrell, Thomas A., Jr.; Leanna, Candice;
 Mathre, Owen.; Rosson, Reinhardt; Running, Jeff;
 Severson, Dave; Song, Linsheng; Wassink, Sarah

PATENT ASSIGNEE(S): Arkion Life Sciences Llc, USA

SOURCE: PCT Int. Appl., 327 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004003175	A2	20040108	WO 2003-US20925	20030701
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				

PRIORITY APPLN. INFO.: US 2002-393348P P 20020701

OTHER SOURCE(S): CASREACT 140:92684

AB A biosynthetic method for producing glucosamine and N-acetylglucosamine is disclosed. Such a method includes the fermentation of a genetically modified microorganism to produce glucosamine and/or N-acetylglucosamine. Also disclosed are genetically modified microorganisms that are useful for

producing glucosamine and N-acetylglucosamine. In addition, methods of recovering N-acetylglucosamine that has been produced by a fermentation process, including methods that result in N-acetylglucosamine of high purity, are described. Also disclosed is a method to produce glucosamine from N-acetylglucosamine.

L18 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2004:3054 CAPLUS

DOCUMENT NUMBER: 140:58538

TITLE: Increased astaxanthin production by *Phaffia rhodozyma* treated with squalene synthase inhibitors

INVENTOR(S): Hoshino, Tatsuo; Masuda, Setsuko; Setoguchi, Yutaka

PATENT ASSIGNEE(S): DSM Ip Assets B.V., Neth.

SOURCE: PCT Int. Appl., 13 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004001057	A1	20031231	WO 2003-EP3742	20030410
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			

PRIORITY APPLN. INFO.: EP 2002-13784 A 20020621

AB The present invention relates to a biol. process for producing carotenoids utilizing a microorganism which is capable of producing carotenoids and belonging to the genus *Xanthophyllomyces* (*Phaffia*) in the presence of an inhibitor for biosynthesis of sterols from farnesyl pyrophosphate. Thus, *Phaffia rhodozyma* ATCC 96594 was cultured in the presence of 5 µg/L of [3-(3-allyl-biphenyl-4-yloxy)-propyl]-isopropylamine. After 7 days cultivation the growth level for *Phaffia rhodozyma* was similar to the untreated control while the astaxanthin concentration was 29% higher.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2003:938237 CAPLUS

DOCUMENT NUMBER: 140:234416

TITLE: Metabolic engineering of the astaxanthin-biosynthetic pathway of *Xanthophyllomyces dendrorhous*

AUTHOR(S): Visser, Hans; van Ooyen, Albert J. J.; Verdoes, Jan C.

CORPORATE SOURCE: Section of Fungal Genomics, Wageningen University, Wageningen, 6703 HA, Neth.

SOURCE: FEMS Yeast Research (2003), 4(3), 221-231

CODEN: FYREAG; ISSN: 1567-1356

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB This review describes the different approaches that have been used to manipulate and improve carotenoid production in *Xanthophyllomyces dendrorhous*.

The red yeast *X. dendrorhous* (formerly known as *Phaffia rhodozyma*) is one of the microbiol. production systems for natural astaxanthin. Astaxanthin is applied in food and feed industry and can be used as a nutraceutical because of its strong antioxidant properties. However, the production levels of astaxanthin in wild-type isolates are rather low. To increase the astaxanthin content in *X. dendrorhous*, cultivation protocols have been optimized and astaxanthin-hyperproducing mutants have been obtained by screening of classically mutagenized *X. dendrorhous* strains. The knowledge about the regulation of carotenogenesis in *X. dendrorhous* is still limited in comparison to that in other carotenogenic fungi. The *X. dendrorhous* carotenogenic genes have been cloned and a *X. dendrorhous* transformation system has been developed. These tools allowed the directed genetic modification of the astaxanthin pathway in *X. dendrorhous*. The *crtYB* gene, encoding the bifunctional enzyme phytoene **synthase**/lycopene cyclase, was inactivated by insertion of a vector by single and double cross-over events, indicating that it is possible to generate specific carotenoid-biosynthetic mutants. Addnl., overexpression of *crtYB* resulted in the accumulation of β -carotene and echinone, which indicates that the oxygenation reactions are rate-limiting in these recombinant strains. Furthermore, overexpression of the phytoene desaturase-encoding gene (*crtI*) showed an increase in monocyclic carotenoids such as torulene and HDCO (3-hydroxy-3',4'-didehydro- β , ψ -carotene-4-one) and a decrease in bicyclic carotenoids such as echinone, β -carotene and astaxanthin.

REFERENCE COUNT: 66 THERE ARE 66 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2003:552334 CAPLUS

DOCUMENT NUMBER: 139:244763

TITLE: Metabolic engineering of the carotenoid biosynthetic pathway in the yeast *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*)

AUTHOR(S): Verdoes, Jan C.; Sandmann, Gerhard; Visser, Hans; Diaz, Maria; van Mossel, Minca; van Ooyen, Albert J. J.

CORPORATE SOURCE: Division of Industrial Microbiology, Department of Agrotechnology and Food Sciences, Wageningen University, Wageningen, Neth.

SOURCE: Applied and Environmental Microbiology (2003), 69(7), 3728-3738

CODEN: AEMIDF; ISSN: 0099-2240

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The *crtYB* locus was used as an integrative platform for the construction of specific carotenoid biosynthetic mutants in the astaxanthin-producing yeast *Xanthophyllomyces dendrorhous*. The *crtYB* gene of *X. dendrorhous*, encoding a chimeric carotenoid biosynthetic enzyme, could be inactivated by both single and double crossover events, resulting in non-carotenoid-producing transformants. In addition, the *crtYB* gene, linked to either its homologous or a glyceraldehyde-3-phosphate dehydrogenase promoter, was overexpressed in the wild type and a β -carotene-accumulating mutant of *X. dendrorhous*. In several transformants containing multiple copies of the *crtYB* gene, the total carotenoid content was higher than in the control strain. This increase was mainly due to an increase of the β -carotene and echinone content, whereas the total content of astaxanthin was unaffected or even lower. Overexpression of the phytoene **synthase**-encoding gene (*crtI*) had a large impact on the ratio between mono- and bicyclic carotenoids. Furthermore, we showed that in metabolic engineered *X. dendrorhous* strains, the competition between the enzymes phytoene desaturase and lycopene cyclase for lycopene governs the metabolic flux either via β -carotene to astaxanthin or via 3,4-didehydrolycopene to 3-hydroxy-3'-4'-didehydro- β , ψ -caroten-4-

one (HDCO). The monocyclic carotenoid torulene and HDCO, normally produced as minority carotenoids, were the main carotenoids produced in these strains.

REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2002:784267 CAPLUS

DOCUMENT NUMBER: 137:261996

TITLE: Prenyl alcohol enhanced manufacture with microorganism in the presence of squalene **synthase** inhibitor

INVENTOR(S): Muramatsu, Masayoshi; Obata, Mitsuo; Shimizu, Akira

PATENT ASSIGNEE(S): Toyota Motor Corp., Japan

SOURCE: Jpn. Kokai Tokkyo Koho, 37 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2002300896	A2	20021015	JP 2002-10528	20020118
PRIORITY APPLN. INFO.:			JP 2001-21547	A 20010130
AB	Squalene synthase inhibitors (I) such as BMS-187745 are useful for enhanced extracellular manufacture of prenyl alcs. with prenyl alc.-producing microorganism such as Saccharomyces. I are also selected from squalene synthase -inhibiting phosphonic acid derivs. (SQAD). The prenyl alcs. are selected from geranylgeraniol, farnesol, and nerolidol.			

L18 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2002:503431 CAPLUS

DOCUMENT NUMBER: 137:78001

TITLE: Microorganisms for production of prenyl alcohol

INVENTOR(S): Muramatsu, Masayoshi; Obata, Shusei; Shimizu, Sakayu

PATENT ASSIGNEE(S): Toyota Jidosha Kabushiki Kaisha, Japan

SOURCE: Eur. Pat. Appl., 60 pp.

CODEN: EPXXDW

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1219704	A2	20020703	EP 2001-130425	20011220
EP 1219704	A3	20030102		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
JP 2002291494	A2	20021008	JP 2001-375842	20011210
US 2003096385	A1	20030522	US 2001-22434	20011220
PRIORITY APPLN. INFO.:			JP 2000-401951	A 20001228
			JP 2001-375842	A 20011210
AB	The present invention provides a high production method of prenyl alc., which comprises culturing prenyl alc.-producing cells in a medium with an increased sugar content in the presence of at least one member selected from the group consisting of a surfactant, a fat or oil, and a terpene to produce and accumulate prenyl alc. in the cells; allowing the accumulated prenyl alc. to be secreted from the cells; and then collecting prenyl alc. The present invention enables prenyl alc. to be highly produced in and effectively secreted from prenyl alc.-producing cells by culturing the cells in a medium with an increased sugar content in the presence of at			

least one member selected from the group consisting of a surfactant, a fat or oil, and a terpene.

L18 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:645717 CAPLUS

DOCUMENT NUMBER: 133:234467

TITLE: Cloning and sequence of astaxanthin **synthase** from *Phaffia rhodozyma* and use of the enzyme for production of astaxanthin

INVENTOR(S): Hoshino, Tatsuo; Ojima, Kazuyuki; Setoguchi, Yutaka

PATENT ASSIGNEE(S): F. Hoffmann-La Roche A.-G., Switz.

SOURCE: Eur. Pat. Appl., 46 pp.

CODEN: EPXXDW

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1035206	A1	20000913	EP 2000-104430	20000303
EP 1035206	B1	20031015		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
US 6365386	B1	20020402	US 2000-518386	20000303
AT 252155	E	20031115	AT 2000-104430	20000303
CN 1266101	A	20000913	CN 2000-103755	20000308
BR 2000001369	A	20010814	BR 2000-1369	20000308
JP 2000262294	A2	20000926	JP 2000-65041	20000309
US 2003077691	A1	20030424	US 2002-66007	20020201

PRIORITY APPLN. INFO.: EP 1999-104668 A 19990309
EP 2000-101666 A 20000201
US 2000-518386 A3 20000303

AB The present invention is directed to genetic materials useful for the preparation of astaxanthin from β -carotene, such as polypeptides having astaxanthin **synthase** activity, DNA fragments coding for astaxanthin **synthase**, recombinant organisms and the like. Those novel genetic materials may be originated from *Phaffia rhodozyma*. Cloning, genomic and cDNA sequences of astaxanthin **synthase** of *P. rhodozyma* and amino acid sequence of the encoded enzyme are disclosed. The present invention also provides a process for the production of astaxanthin.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1999:796836 CAPLUS

DOCUMENT NUMBER: 132:147435

TITLE: Isolation and functional characterization of a novel type of carotenoid biosynthetic gene from *Xanthophyllomyces dendrorhous*

AUTHOR(S): Verdoes, J. C.; Krubasik, P.; Sandmann, G.; Van Ooyen, A. J. J.

CORPORATE SOURCE: Division of Industrial Microbiology, Department of Food Technology and Nutritional Sciences, Wageningen University, Wageningen, 6700 EV, Neth.

SOURCE: Molecular and General Genetics (1999), 262(3), 453-461
CODEN: MGGEAE; ISSN: 0026-8925

PUBLISHER: Springer-Verlag

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The red heterobasidiomycetous yeast *Xanthophyllomyces dendrorhous* (perfect state of *Phaffia rhodozyma*) contains a novel type of carotenoid biosynthetic enzyme. Its structural gene, designated crtYB, was isolated

by functional complementation in a genetically modified, carotenogenic *Escherichia coli* strain. Expression studies in different carotenogenic *E. coli* strains demonstrated that the *crtYB* gene encodes a bifunctional protein involved both in synthesis of phytoene from geranylgeranyl diphosphate and in cyclisation of lycopene to β -carotene. By sequence comparison with other phytoene **synthases** and complementation studies in *E. coli* with various deletion derivs. of the *crtYB* gene, the regions responsible for phytoene synthesis and lycopene cyclisation were localized within the protein.

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1997:506702 CAPLUS

DOCUMENT NUMBER: 127:145923

TITLE: Improved transformation of and expression in *Phaffia* by using the promoter of glycolytic pathway gene or ribosomal protein gene

INVENTOR(S): Verdoes, Jan Cornelis; Wery, Jan

PATENT ASSIGNEE(S): Gist-Brocades B.V., Neth.; Ooijen, Albert Johannes Joseph; Verdoes, Jan Cornelis; Wery, Jan

SOURCE: PCT Int. Appl., 117 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9723633	A1	19970703	WO 1996-EP5887	19961223
W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
EP 780474	A1	19970625	EP 1995-203620	19951222
R: NL				
CA 2241267	AA	19970703	CA 1996-2241267	19961223
AU 9713087	A1	19970717	AU 1997-13087	19961223
AU 725340	B2	20001012		
EP 870042	A1	19981014	EP 1996-944694	19961223
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2000507087	T2	20000613	JP 1997-523340	19961223
US 6329141	B1	20011211	US 1998-91725	19981119
PRIORITY APPLN. INFO.:			EP 1995-203620 A	19951222
			EP 1996-200943 A	19960411
			WO 1996-EP5887 W	19961223

AB The transformation efficiency of and expression level in *Phaffia* can be improved by using the high-level promoter of a glycolytic pathway gene (e.g. glyceraldehyde-3-phosphate dehydrogenase (gpd)) or a ribosomal protein gene (e.g. 40S ribosomal protein S27). High level expression of a carotenoid biosynthetic pathway gene in *Phaffia rhodozyma* may be obtained by using an expression vector containing one of the above promoters and the gene *gpd* terminator/polyadenylation site. Also disclosed are the cDNA encoding the enzymes involved in the carotenoid biosynthetic pathway of *Phaffia rhodozyma*: isopentenyl diphosphate isomerase (*idi*), geranylgeranyl pyrophosphate **synthase** (*crtE*), phytoene **synthase** (*crtB*), phytoene desaturase (*crtI*), and lycopene cyclase (*crtY*). Heterologous expression of carotenogenic genes from *Erwinia*

uredovora in *P. rhodozyma* by using an expression vector containing the gene *gpd* promoter was demonstrated. Isolation of carotenogenic genes by heterologous hybridization from carotenogenic fungi was also shown. Use of the vectors, transformed *P. rhodozyma* for making proteins and/or carotenoids (e.g. astaxanthin), and methods for isolating highly expressed promoters from *Phaffia* are also claimed.

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(FILE 'HOME' ENTERED AT 13:27:04 ON 15 APR 2004)

FILE 'CAPLUS' ENTERED AT 13:27:12 ON 15 APR 2004

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L1          1 S ASTAXANTHIN SYNTHASE
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L3          1 S E2
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            SET SMA LOGIN
L4          1 S E3
L5          78943 S SYNTHASE
L6          63109 S ACTIVE SITE
L7          1859 S L5 AND L6
L8          17192 S BETA CAROTENE
L9          0 S L7 AND L8
L10         2097 S ASTAXANTHIN
L11         854 S L8 AND L10
L12         1 S L1 AND L11
L13         10 S AST GENE
L14         0 S L5 AND L13
L15         0 S L8 AND L14
L16         337 S RHODOZYMA
L17         78949 S SYNTHASE?
L18         9 S L16 AND L17

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